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# ANEMIA OF CENTRAL ORIGIN

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## Abstract

Hypoproliferative anemia results from the inability of bone marrow to produce adequate numbers of red blood cells. The list of conditions that cause hypoproliferative anemia is long, starting from common etiologies as iron deficiency to rarer diagnoses of constitutional bone marrow failure syndromes. There is no perfect diagnostic algorithm, and clinical data may not always clearly distinguish “normal” from “abnormal”, yet it is important for practicing clinicians to recognize each condition so that treatment can be initiated promptly. This review describes diagnostic approaches to hypoproliferative anemia, with particular emphasis on bone marrow failure syndromes.

## INTRODUCTION

Anemia of central origin, or hypoproliferative anemia, broadly refers to anemia resulting from underproduction of red blood cells by the bone marrow. Hypoproliferative anemia is characterized by an inappropriately low reticulocyte count and is distinguished from anemia secondary to blood loss or peripheral erythrocytes destruction, which are accompanied by elevated reticulocyte counts from a bone marrow regenerative response. Table 1 lists a classification of hypoproliferative anemia. The most common etiology worldwide is iron deficiency, followed by the anemia of chronic disease and inflammation, and the anemia of renal disease (1) (the anemia of chronic disorders is discussed by Weiss in this issue).

Clinical reasoning toward a diagnosis often employs at least two methods: pattern recognition or clinical gestalt and algorithms that use decision trees to differentiate causes or associations. Clinical gestalt can be described as pattern recognition of a patient's presentation as a whole, and physicians use it to quickly reach a tentative clinical decision. For example, a low hemoglobin in a young woman with no known medical issues except for menorrhagia and recent pregnancy “looks like iron deficiency”, based on a coherent

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conception of a typical iron deficiency patient. Pattern recognition improves with clinical experience (2). However, clinical gestalt is subject to bias and error, as in “path-determination”, in which inconsistent or incompatible features are ignored to the patient's peril. Parallel to the process of the pattern recognition is a review of a nonheuristic range of didactic aids, from lists of differential diagnoses to decision trees and formal algorithms, some now computer assisted. Differential diagnoses can be dynamically modified and shortened in an efficient manner by systematically incorporating preliminary clinical data, as well as clinical gestalt.

This review aims to provide helpful information for practicing physicians to refine their both pattern recognition and differential diagnosis of hypoproliferative anemia, particularly of bone marrow failure syndromes. We start with a brief overview of general diagnostic considerations followed by sections dedicated to representative bone marrow failure syndromes. Detailed pathophysiologies and treatments for specific bone marrow failure syndromes have recently been reviewed (3-8) and are not discussed in detail here. Myelodysplastic syndrome (MDS), another important category of bone marrow failure, is discussed by Santini in this issue.

## DIAGNOSTIC APPROACH TO HYPOPROLIFERATIVE ANEMIA

### History and Physical Examination

A careful history is critical to develop insight into both the underlying causes of anemia and the potential role of concurrent illnesses. Any history suggestive of blood loss should be elicited by asking specific pertinent questions. Social history should be reviewed, especially for exposure to toxic substances (occupational exposure, smoking, alcohol, etc.) and risk of chronic infection such as human immunodeficiency virus (HIV), hepatitis viruses, and tuberculosis. A dietary history may suggest nutritional deficiencies are the dominant cause of hypoproliferative anemia. Family history is important, not only in pediatric patients but also in adults, as some inherited anemias can present late in life without apparent physical anomalies, and an affected pedigree may be the only clue to a constitutional origin of blood abnormalities. Physical findings of anemia are usually non-specific, such as pallor and systolic murmurs. However, a systematic physical examination can provide valuable clues to underlying etiologies: neurologic abnormalities in vitamin B12 deficiency, hepatosplenomegaly and lymphadenopathy in lymphoproliferative disease, spoon-shaped nails (koilonychia) and angular stomatitis in iron deficiency, and specific physical anomalies associated with constitutional bone marrow failure syndromes (see below).

### Laboratory Evaluation

Considerable information is contained in the initial laboratory tests that led to the detection of anemia: complete blood count (CBC) with white blood cell (WBC) differential, mean corpuscular volume (MCV), and reticulocyte count. Further basic laboratory tests are performed as appropriate depending on the clinical context: complete metabolic panel, lactate dehydrogenase (LDH), haptoglobin, iron study, occult blood, vitamin B12, folate, and thyroid function tests. If inherited hemoglobinopathies are suspected based on ethnicity and family history, electrophoresis of hemoglobin should be performed. The presence of

abnormalities in other hematopoietic cell lines alters the differential diagnosis of hypoproliferative anemia, and identification of red blood cell size is also useful (see Iolascon – microcytic anemia, Green – macrocytic anemia, in this issue). More than one etiology may coexist, leading to a mixed picture, so overreliance on preliminary categorizations based on the MCV and other initial test results should be avoided. Examination of the peripheral blood smear confirms the findings of automated counts (CBC), and can reveal schistocytes, teardrop cells, target cells, nucleated red blood cells, as well as morphological changes in WBC and platelets.

The reticulocyte count, a marker of effective erythropoiesis, is the single blood test most important for distinguishing hypoproliferative anemia from other causes (Figure 1). The reference range for the absolute reticulocyte count (ARC) is typically 20-90 K/uL and may vary according to measurement methods (9). When the reticulocyte count is reported as reticulocyte percentage, the absolute reticulocyte count can be computed by multiplying the reticulocyte percentage by the number of red blood cells. Alternatively, adjustment for the degree of anemia is accomplished as the corrected reticulocyte percentage (= reticulocyte percentage x patient's hematocrit [Hct] / reference Hct). The stressed bone marrow releases reticulocytes prematurely into the peripheral blood, where they remain in circulation, referred to as the reticulocyte maturation time. The reticulocyte maturation time is approximately 1 day for a Hct of 45%, 1.5 days for Hct of 35%, 2 days for Hct of 25%, and 2.5 days for Hct of 15% (10). The reticulocyte index accounts for the maturation time and the degree of anemia, and is calculated as reticulocyte index = corrected reticulocyte percentage / maturation time of reticulocytes in peripheral blood in days. A reticulocyte index higher than 2-3 is not consistent with the diagnosis of hypoproliferative anemia (9).

Interpretation of vitamin B12 and folate levels can be difficult. A fasting serum cobalamin level of around 200 pg/mL is often used as a lower normal limit. However, patients with true vitamin B12 deficiency may present with higher serum cobalamin levels (11), and a sensitivity as low as 0.40 has been reported when a serum cobalamin level of <300 pg/mL is used as the diagnostic threshold (12). Total serum cobalamin levels also poorly reflect metabolically active levels of vitamin B12. Only transcobalamin II-bound cobalamin is bioavailable, and it comprises only approximately 20% of total serum cobalamin, and remaining serum cobalamin is bound to haptocorrin and metabolically unavailable (12). Changes in the level of these cobalamin-binding proteins can affect the measured level of cobalamin, for instance, the haptocorrin level can be falsely low in patients with multiple myeloma, while in patients with myeloproliferative disease, reported serum cobalamin levels may be falsely high (11, 13). Serum folate levels only reflect folate intake over the preceding few days, and a low value (less than approximately 3 ng/mL) does not necessarily indicate more meaningful deficiency at the tissue level (11). Red cell folate levels better reflect folate stores for the 3 months prior to testing (1, 14). Methylmalonic acid (MMA) and homocysteine (Hcy) levels measure vitamin availability at the tissue level, and are more reliable indicators of vitamin deficiencies (11). In general, MMA is elevated only in cobalamin deficiency, while Hcy is less specific and can be elevated in both cobalamin and folate deficiencies as well as in vitamin B6 deficiency, hypothyroidism, methotrexate therapy, phenytoin therapy, and in methylenetetrahydrofolate reductase deficiency and other genetic defects (11, 15, 16). Both MMA and Hcy can be high in renal insufficiency (11).

Elevation in plasma MMA and Hcy levels precedes decreased plasma cobalamin and folate levels, respectively. The sensitivity of an elevated plasma MMA is >90% for detecting vitamin B12 deficiency with hematologic abnormalities (17). Although MMA and Hcy tests are more expensive than are standard plasma vitamin levels, a cost-benefit analysis indicated that measurement of MMA is justified in patients with serum cobalamin levels between 80-120 pg/mL and 270-300 pg/mL (12).

If primary bone marrow abnormalities are suspected or when the diagnosis remains uncertain after initial evaluation, bone marrow examination and further specific hematological tests are indicated to confirm the hypoproliferative nature of bone marrow and to identify other underlying pathologies: leukemia, MDS, myeloma, lymphoma, myelofibrosis, or infiltration by malignancy or granuloma (Figure 1, Figure 2). A core biopsy of adequate size and quality should be obtained because bone marrow cellularity and pathological findings may vary depending on the site (Figure 3-A), and underlying pathology may be distorted by crush artifact or handling error. At National Institutes of Health (NIH), a minimum bone marrow core size of at least 1.5 cm is sought when considering a diagnosis of bone marrow failure. In addition, a good quality bone marrow aspirate should contain macroscopically identifiable “spicules” or particles of bone marrow. An aspirate sample without spicules may indicate contamination by peripheral blood. Further specialized hematological studies, such as flow cytometry, cytogenetics, and molecular studies, will be discussed in the following disease-specific sections.

## HYPOPROLIFERATIVE ANEMIA AS A COMPONENT OF BONE MARROW FAILURE SYNDROMES

The possibility of bone marrow failure syndromes increases once other etiologies of hypoproliferative anemia have been excluded (Table 1, Figure 2). Cytopenia can be limited to a single lineage or can be any combination of bicytopenia or pancytopenia. Bone marrow failure can be constitutional or acquired.

### Isolated anemia: PRCA

Pure red cell aplasia (PRCA) is characterized by hypoproliferative anemia in the absence of abnormalities in other hematopoietic lineages. Patients present with symptomatic anemia. Regardless of classification, patients with PRCA share the same characteristic peripheral blood and bone marrow findings of normocytic or macrocytic anemia, profound reticulocytopenia, and severely diminished marrow erythroid precursor cells (Figure 3B-C). Classification of PRCA is shown in Table 2. Age at presentation, family history, and physical anomalies help distinguish constitutional from other forms. Congenital PRCA or Diamond-Blackfan anemia (DBA) is diagnosed at birth or within the first year of life in more than 90% of cases (18), although there are rare reports of presentation in adulthood (19). PRCA in adults are mostly acquired in the setting of underlying infections, autoimmune diseases, or malignancies. Self-limited forms of PRCA include transient aplastic crisis that affects patients with underlying hemolytic anemia following acute parvovirus infection, and transient erythroblastopenia of childhood that occurs in otherwise normal children triggered by yet unidentified viral infections.

**Diamond-Blackfan anemia (DBA)—**Congenital PRCA or Diamond-Blackfan anemia (DBA) is caused by haploinsufficiency of a ribosomal protein gene. Approximately 60% of DBA cases have identifiable mutations in ribosomal genes (20), among which the *RPS19* is most commonly mutated, accounting for about a quarter of DBA cases (21). DBA is inherited in an autosomal dominant pattern, but penetrance and phenotype are variable (20, 22, 23). The severity of DBA varies from in utero complications (preeclampsia, in utero fetal death, in utero growth retardation, hydrop fetalis) (24) to first symptoms of anemia later in life. Thirty to 40% of DBA patients have congenital physical anomalies (21); craniofacial abnormalities are most common and seen in about half of patients, followed by skeletal (commonly malformation of thumbs and upper limb), genitourinary, and cardiac abnormalities (18). Erythrocyte adenosine deaminase (eADA) (25) and hemoglobin F expression are classically increased (26). Genetic sequencing of known ribosomal gene mutations is commercially available, and a positive result supports the diagnosis of DBA (27). Screening for Fanconi anemia with chromosomal breakage analysis and exclusion of other constitutional bone marrow failure syndromes should be considered (discussed later).

Corticosteroids, typically prednisone at the starting dose of 2mg/kg/day (27, 28), are the mainstay of treatment for DBA, with an initial response rate of approximately 80% (29). Once an adequate response is achieved, steroids are slowly tapered (21, 27). However, relapse is frequent and there are insufficient data to support any specific steroid tapering schedule. Since a response is expected within the first few weeks, steroids should be discontinued for non-responders after a maximum of four weeks of administration (21, 27). Hematopoietic stem cell transplantation (HSCT) is the only curative treatment option for DBA, with a 5-year overall survival of approximately 70% for matched sibling donor transplant. The outcome of alternative donor HSCT has substantially improved over the past decade (27). Regardless of prior treatment, one fifth of patients in the DBA registry achieved remission, defined as an adequate hemoglobin level maintained for 6 months or more without any treatment. Overall actuarial survival is approximately 75% at 40 years of age (29).

**Transient aplastic crisis and transient erythroblastopenia of childhood—**Presentation with acute worsening of anemia in children with underlying hemolytic anemia should raise the concern for transient aplastic crisis (acute B19 parvovirus infection), while sudden onset of severe anemia in previously well children points toward transient erythroblastopenia of childhood (no known infectious etiology). Anemia in children may have different manifestations compared to adults, such as failure to thrive, poor appetite, or apathy. Transient aplastic crisis resolves spontaneously within 1 to 2 weeks of infection, with the appearance of neutralizing antibodies to B19 parvovirus (30, 31). In contrast, it may take a few weeks to months before resolution of transient erythroblastopenia of childhood (32). In addition to reticulocytosis, hemoglobin, white cell, and platelet numbers may temporarily rise to higher than normal values during the process of bone marrow recovery.

**Acquired PRCA—**Acquired PRCA develops predominantly in adults, and is caused by antibody- and/or cellular-mediated inhibition of erythropoiesis. Evaluation for possible causes and associated concurrent conditions is important, as listed in Table 2. Acquired

PRCA is pathophysiologically and clinically associated with autoimmune diseases and malignancies (such as chronic lymphocytic leukemia [CLL], large granular lymphocytic leukemia [LGL leukemia] and thymoma) (33-37). Other causes of acquired PCRA are persistent B19 parvovirus infection in the setting of underlying immunodeficiency (such as acquired immunodeficiency syndrome [AIDS], immunosuppressant recipients) (38-40), antierythropoietin antibodies secondary to admininstration of recombinant human erythropoietin (41-43), pregnancy (44), and major ABO mismatched hematopoietic stem cell transplantation (45). There are numerous drugs and other conditions associated with PRCA, but causation is less well established (1, 43, 46).

Acquired PRCA secondary to persistent B19 parvovirus infection is effectively treated with immunoglobulin infusion (47, 48). For immune-mediated acquired PRCA, various immunosuppressive therapies are employed. Historically, corticosteroids were the first treatment of choice (7), with response rates of approximately 40% (49). However, relapses frequently occurred during steroid tapering, and complications of long-term steroid treatment became problematic. More recently, cyclosporine (CsA) has been advocated as the first treatment choice, and response rate to CsA monotherapy is approximately 70-80% (50, 51). Relapses are also frequent after CsA discontinuation (51), and the reported relapse-free period after discontinuation is 10 months (range 1.5 to 40 months) (51). Cytotoxic drugs are usually reserved for CsA-refractory disease or for patients with contraindications to CsA (7). Cyclophosphamide may offer better response than CsA for LGL leukemia-associated PRCA (52, 53). Several reports have shown that some patients with refractory PRCA with or without underlying lymphoproliferative disease can be successfully treated with alemtuzumab, an anti-CD52 monoclonal antibody (54, 55). Other treatment options include antithymocyte globulin (ATG) (56, 57) and rituximab (58, 59). For thymoma-related PRCA, the hematological response rate after thymectomy is 25-30% at best (60, 61). Thymoma-associated PRCA responds to CsA monotherapy or CsA-containing regimens (62). Matched sibling HSCT has been performed successfully for refractory cases (63-65).

## Anemia as a component of bicytopenia or pancytopenia

**Acquired aplastic anemia—**The definition of aplastic anemia (AA) is reduced blood counts and a hypocellular bone marrow replaced with fat (Figure 3D). Radiation, chemical exposure (benzene), drugs, infection, and pregnancy have all been historically linked to the development of aplastic anemia (46). However, it is often difficult to distinguish association from causation, and the majority of acquired AA cases remain idiopathic (3). Immune-mediated mechanisms appear to be responsible for the severe deficiency of hematopoietic stem and progenitor cells (HSPC) (66), inferred from the success of immunosuppressive therapy (IST) in the majority of patients (67-70). Research laboratory studies also support underlying immune processes, including the demonstration of expansion of cytotoxic T-cells expressing type 1 cytokines and subsequent marrow suppression (71-74), regulatory T-cell deficiency (75, 76), acquired mutations in STAT3 and clonal cytotoxic T-cells in a subset of AA (77), and animal models (78).

Symptoms due to anemia and thrombocytopenia (typically mucocutaneous hemorrhage, petechia, and menorrhagia) are the most common reasons for patients to seek medical care,

while infection is an uncommon initial presentation even in the setting of severe aplastic anemia (SAA) (3). SAA has been defined as a hypocellular marrow for age and at least two of the following criteria: absolute neutrophil count <500/uL, absolute reticulocyte count <60,000/uL (or corrected reticulocyte count (CRC) < 1%), and platelet count <20,000/uL (3, 79). Over 40% of patients with non-severe AA present without any symptoms (80), but pancytopenia worsens over time into the severe range in about half of these cases. The bone marrow is hypocellular without overt dysplasia or increase in blasts or other evidence of MDS and leukemia. The initial clinical presentation and bone marrow morphology of hypocellular MDS can be similar to aplastic anemia, and the distinction between these two entities can be difficult. Modest dyserythropoietic and megalobastic changes in red blood cells may be seen in AA, but dysplastic findings in megakaryocytes (especially small and mononuclear megakaryocytes) favor the diagnosis of MDS (Figure 3E-H). Quantification of CD34+ cells may also help distinguish the two conditions: low percentages of CD34+ cells (<0.5%) are associated with AA and higher CD34+ (>= 1%) may be indicative of hypocellular MDS (81). Abnormal cytogenetics results alone do not automatically exclude AA unless the abnormalities are MDS-defining. For some specialists, abnormalities trisomy 8, trisomy 6, and trisomy 15 are accepted in AA cases upon presentation, but the clinical relevance of these abnormalities remains controversial (82-84). Per protocol at NIH, we exclude patients with cytogenetic abnormalities from clinical trials of AA. Patients with AA can gain new cytogenetic abnormalities over time, and specific high-risk abnormalities (especially loss of chromosome 7) are associated with progression to MDS or leukemia (85).

Overlap of paroxysmal nocturnal hemoglobinuria (PNH) with AA is common and identified in approximately 50% of cases (the AA/PNH syndrome) (86, 87). Peripheral blood cell surface flow cytometry for expansion of glycophosphoinositol (GPI) -anchored proteins should be performed to identify and quantify a PNH clone. Whether the presence of PNH clone is a prognostic indicator for a better response to IST is unsettled (86, 88-90). Our retrospective analysis of over 200 AA cases suggested that specific measures to address clinical PNH were rarely required (<5% of the cohort) (86). Patients without a PNH clone at presentation usually do not develop a clone following IST, and clones may disappear over time in some cases. Clinical signs and symptoms of PNH, such as hemolysis or thrombosis, were rarely observed among AA/PNH syndrome patients who underwent IST unless a large clone (>50%) persisted over time (86). Regardless of the PNH clone size at presentation, initial treatment should be immunosuppression or transplant to address underlying marrow failure (3).

Definitive treatment should be initiated promptly for severe aplastic anemia (SAA) to avoid the risk of serious infectious and other complications. Patients with non-severe AA can be monitored, particularly when they do not require transfusion, because it is unknown whether definitive treatment has long-term benefit for milder pancytopenia. Definitive treatment for SAA is either HSCT or IST. HSCT is a curative option, and HSCT is preferred for younger patients (e.g. <40 year-old) with a human leukocyte antigen (HLA)-matched sibling donor (3) (91-93). The risks of graft-versus-host disease (GVHD), related morbidity, and mortality increase with age (94-96). Upfront matched-related donor transplant in children has an excellent outcome, and 3-year overall survival and 3-year event free survival are both around 90% (97). In SAA, bone marrow is the preferred stem cell source because peripheral blood

stem cell transplantation (PBSCT) is associated with higher risk of chronic GVHD and mortality (98-100). The outcome of matched unrelated donor (MUD) transplantation is improving, and MUD transplantation is recommended for younger patients who do not have a matched sibling and have failed IST, but generally not as first-line therapy (3, 101).

IST is initial therapy for most patients when HSCT is not a feasible option due to lack of suitable donors, age, or comorbidities. The combination of horse antithymocyte globulin (ATG) and cyclosporine (CsA) is standard (102), with a response rate of 60-70% and overall long-term survival comparable to HSCT (67-70). Baseline ARC and absolute lymphocyte count (ALC) have prognostic implications: patients with a higher baseline ARC (≥ 25K/uL) and ALC (≥ 1000/uL) have better response to IST and better survival compared to lower baseline ARC/ALC group (response rate to IST at 6 months, 83% vs. 41% [$p<0.001$], and 5-year survival, 92% vs. 53% [$p<0.001$]) (90).

Recently, an oral thrombopoietin mimetic, eltrombopag was approved by the United States Food and Drug Administration (FDA) for the treatment of SAA. In refractory SAA, eltrombopag monotherapy provided hematological responses in at least one lineage in almost half of study patients at 12 weeks, with many achieving multilineage hematological improvements and transfusion independence (103). Long-term follow-up of the study cohort revealed that improvements were sustained even after discontinuation of eltrombopag (104). Currently, ongoing clinical trials at the NIH are testing the efficacy and safety of eltrombopag in combination with standard IST as upfront therapy. Eltrombopag is generally well tolerated with minimal side effects, but long-term effects of the treatment, including clonal evolution, are yet to be determined. Uncontrolled studies have shown that androgens provide sustained hematological recoveries and survival benefit in some patients (105, 106).

**Stereotypical constitutional bone marrow failure syndromes that present in adults—**Bone marrow findings of constitutional syndromes are identical to acquired AA (3). Approximately 30% of pediatric bone marrow failures are comprised of constitutional (or inherited) syndromes (46). Some constitutional bone marrow failure syndromes can present in adulthood even without suggestive family history (Table 3). When bone marrow failure syndromes are suspected, it is important to exclude constitutional conditions in appropriate settings because it has important therapeutic implications.

**Fanconi Anemia:** Fanconi anemia (FA), the most common constitutional bone marrow failure syndrome, is inherited in an autosomal recessive or X-linked recessive manner (46). There are at least 16 known FA genes, which encode proteins essential for DNA repair and genomic stability (107). FA is associated with a predisposition for both hematologic and solid malignancies. Bone marrow failure is the most common first hematopoietic presentation of FA (107). Classically, FA is associated with congenital physical anomalies, such as skin hyperpigmentation, short stature, upper limb anomalies, skeletal changes, hypogonadism, renal malformation, and characteristic facial features (8). However, the manifestations of FA are heterogeneous, and up to one third of patients lack these physical features (108). The median age at diagnosis is 6.5 years, but varies widely from 0 to 49 years (8). The cumulative incidence of bone marrow failure, hematologic neoplasms, and non-hematologic neoplasms by 40 years of age is reported to be 90%, 33%, and 28%,

respectively (108). Thus, it is reasonable to screen for FA in patients who present with bone marrow failure syndromes up to at least 40 years of age even in the absence of a suggestive family history or physical anomalies.

Increased chromosomal breakage of peripheral blood cells after exposure to the DNA crosslinking agents, diepoxybutane (DEB) or mitomycin C (MMC), is diagnostic of FA. Occasionally when results in peripheral blood cells are normal and there is a high clinical suspicion for FA, chromosome breakage analysis of cultured skin fibroblasts is performed (6). Distinction between acquired AA and FA has important clinical implications: HSCT is the only curative treatment and IST is futile for FA. Because of sensitivity to DNA damages inherent in FA, HSCT regimens require modification and vigilant monitoring for malignancies is important. Moreover, genetic screening of family members is necessary before considering them as potential stem cell donors and for genetic counseling.

**Telomere Diseases: Dyskeratosis Congenita in Children and Telomeropathies in Adults:** Dyskeratosis congenita is another classical example of constitutional bone marrow failure that usually manifests early in life. Dyskeratosis congenita shares some clinical features with FA, including early development of bone marrow failure, associated physical anomalies, and predisposition to cancers (5). The classic mucocutaneous triad is patchy skin hyperpigmentation, dystrophic nails, and oral leukoplakia. Skin and nail changes typically present during first decade of life (109). Although mucocutaneous manifestations are highly prevalent (110), the complete mucocutaneous triad is seen in less than half of patients (46). Bone marrow failure develops by the third decade of life (median age of onset 8 years old) (110), and is the major cause of mortality. Classical X-linked dyskeratosis congenita is caused by mutations in the *DKC1* gene. *DKC1* encodes a protein called dyskerin (111), which is a part of the telomere repair complex (or telomerase), and loss of its function destabilizes the complex, leading to an inability of cells to maintain telomeres (4, 5). The telomere repair complex functions to maintain telomeres in cells with high replicative capacity, such as hematopoietic stem cells, preventing chromosome erosion, cell senescence, and genomic instability (5). Mutations of genes that encode components of telomerase result in accelerated telomere attrition and very short telomere length (112-114). Genetic studies in pedigrees with autosomal dominant and recessive patterns have revealed gene mutations in various components of the telomere repair complex or shelterin (the telomere protection complex), including *TERC* (a RNA template for telomerase) (115, 116), *TERT* (a reverse transcriptase) (117), and, much less frequently, *NOP10* (118), *NHP2* (119), and *TINF2* (120).

The distinction between constitutional and acquired forms of aplastic anemia is blurred by recent advances in the understanding of telomere biology and its role in hematopoietic stem cell functions. Some acquired aplastic anemia cases among adults without associated physical anomalies or a suggestive family history have *TERC* and *TERT* mutations (115, 117). In addition to bone marrow failure, telomeropathies cause pulmonary fibrosis (121) and liver disease such as cryptogenic cirrhosis and portal hypertension (122). Premature graying of hair is a features of telomeropathies and a helpful clue in the clinic (4). *TERC* and *TERT* mutations have highly variable penetrance, and they are considered as genetic risk factors that modify the susceptibility of the host to environmental insults rather than genetic

determinants of organ failure (5). For example, smoking appears to accelerate progression of pulmonary fibrosis in patients known to have *TERT* mutations (123).

Average chromosomal telomere content in peripheral blood leukocytes can be measured with commercial assays, such as standardized flow-FISH (fluorescent in situ hybridization with labeled probes of telomere repeats measured by flow cytometry) and quantitative polymerase chain reaction (qPCR) amplification for telomere DNA (Figure 4) (124, 125). Results must be adjusted for age, because telomere length normally declines over a lifespan in healthy individuals. Telomere length of total lymphocytes below the first percentile for age strongly supports the diagnosis of telomere disease with high sensitivity and specificity , while telomere content assayed in total leukocytes is less specific (126). However, telomere length above the first percentile does not exclude the diagnosis, as some patients with confirmed mutations in telomerase genes can have normal telomere length (127). Patients with other inherited bone marrow failure syndromes (e.g. FA, DBA, and Shwachman-Diamond syndrome) as well as patients with acquired AA without identifiable mutations can also have short telomeres (114, 128).

The only potentially curable treatment is HSCT, but some patients respond to immunosuppressive therapies as in acquired AA. As in FA, family members must be confirmed to lack mutations before they can serve as donors. Transplant with reduced intensity conditioning regimen has been successfully performed for dyskeratosis congenita patients (129, 130). Androgens increase *TERT* gene expression and telomerase enzymatic activity (131), and improve hematological findings in about half of the patients (105, 132).

**GATA2 deficiency:** Haploinsufficiency of the hematopoietic transcription factor GATA2 results in a range of hematologic syndromes (133). GATA2 deficiency can be sporadic or inherited in an autosomal dominant pattern (134, 135). Most mutations occur in the second zinc finger domain or a conserved intronic enhancer element of *GATA2*, but patients can also have uniallelic expression of GATA2 without an identifiable mutation (133). GATA2 deficiency causes familial MDS / AML (136), monocytopenia and mycobacterial infection (monoMAC syndrome) (135), dendritic cell, myeloid, and NK cell lymphopenia (DCML) (137), Emberger syndrome (lymphedema and MDS) (138), idiopathic bone marrow failure syndromes (139) and aplastic anemia (140). Non-hematological clinical features include susceptibility to nontuberculous mycobacteria (NTM) and human papilloma viruses (HPVs), warts, panniculitis, erythema nodosum, lymphedema, pulmonary complications (including alveolar proteinosis, which is exceedingly rare in general populations), and sensorineural hearing loss (133, 134, 141). The frequency of GATA2 mutations in bone marrow failure syndromes is not well established. In an NIH cohort with a confirmed diagnosis of acquired aplastic anemia, GATA2 mutations were identified in approximately 5% of patients (140). The natural history of bone marrow failure associated with GATA2 mutations can be atypical: Figures 5A-G show peripheral blood and bone marrow findings from a patient who initially presented with pancytopenia and marrow findings consistent with AA, which rapidly evolved into AML with myelodysplastic changes within two years.

As in other constitutional bone marrow failure syndromes, identification of GATA2 mutations has clinical implications, especially for screening of family donors for the same

genetic defects and to recognize increased risks of multi-organ dysfunction associated with the mutations. Flow cytometry of peripheral blood and bone marrow can distinguish GATA2 deficiency from idiopathic AA: GATA2 deficiency is associated with disproportionately reduced numbers of peripheral blood monocytes, B-cells, and NK cells. The bone marrow from patients with GATA2 deficiency also is characterized by markedly reduced monocytes, B-cells, and NK cells, as well as by the absence of hematogones (142) (Figure 6).

## MYELOPHTHISIC ANEMIA

Myelophthisic anemia is a broad and antique term used to describe hypoproliferative anemia resulting from bone marrow fibrosis and infiltration by abnormal tissues. Myelophthisic anemia may be a manifestation of primary myelofibrosis or fibrosis secondary to other conditions (Table 1). Primary myelofibrosis (PMF) is a clonal myeloproliferative disease, characterized by bone marrow fibrosis, hepatosplenomegaly, extramedullary hematopoiesis, ineffective erythropoiesis, and abnormal cytokine expressions. PMF is classified as one of the myeloproliferative neoplasms (MPNs), along with polycythemia vera (PV) and essential thrombocythemia (ET). Patients commonly present with constitutional symptoms such as fever, night sweats, fatigue, weight loss, pruritis, bone pain, and symptoms related to extrameduallary hematopoiesis (discomfort or pain from splenomegaly, early satiety). Rarely, bleeding or thrombosis can be the presenting symptom. About one-fourth of patients are asymptomatic (143). Janus kinase (JAK) 2 mutations (most commonly V617F) are seen in approximately 60% of patients with PMF or post-ET MF, and are more prevalent among patients with post-PV MF (95%) (144-146).

Secondary processes must be excluded before making the diagnosis of primary myelofibrosis. Other hematological disorders that can be accompanied by bone marrow fibrosis include multiple myeloma, lymphomas, hairy cell leukemia, AML, mastocytosis, and many others (46). Reactive myelofibrosis may occur due to infiltrating metastatic cancers (especially breast, lung, and prostate), disseminated mycobacterial infection or infection with other organisms (1, 46). Myelofibrosis has been associated with autoimmune diseases (147, 148), granulomatous diseases like sarcoidosis (149), and conditions related to bone metabolism, such as renal osteodystrophy (150), hypo- and hyperparathyroidism, vitamin D deficiency, and Paget disease (46).

The peripheral blood smear shows teardrop red blood cells with leukoerythroblastic features, characterized by appearance of immature myeloid cells and nucleated erythrocytes. Bone marrow aspiration is often difficult and a “dry tap” in more than half of cases (151). Treatment and prognosis depends on the etiology of myelofibrosis. Treatable causes must be recognized, because addressing the primary disorder, such as infection or autoimmune disease, may improve marrow fibrosis (147, 148, 152). The choice of treatment for primary myelofibrosis is determined in a risk-adaptive manner. Most experts agree on “wait-and-see” or symptom-guided approach for lower risk disease (153, 154).

## TRANSFUSION AND SUPPORTIVE CARE

In all bone marrow failure syndromes, adequate transfusion of blood products is important to correct associated symptoms and prevent organ dysfunction. In general, red blood cell

transfusions are provided to maintain a hemoglobin above 7 g/dL (higher than 9 g/dL for patients with ischemic heart disease) (3). Platelet transfusion to maintain a count of 10 K/uL is a routine to avoid spontaneous severe bleeding in stable patients. All red blood cell and platelet products should be leukoreduced to minimize the risk of HLA alloimmunization and resultant transfusion refractoriness, which is problematic for long-term transfusion support. HLA alloimmunization may limit availability of suitable donors and negatively impact transplant outcomes (155, 156). Leukoreduction of blood products also decreases the risk of febrile transfusion reaction and transfusion-related transmission of cytomegalovirus (CMV) (157). Profoundly immunocompromised patients, especially recipients of HSCT, are at risk for the lethal complication of transfusion-associated GVHD (158). The risk of transfusion-associated GVHD can be eliminated by irradiation of blood products (159), although irradiation of blood products is not proven necessary in uncomplicated SAA. The use of G-CSF or erythropoietin is generally ineffective in severe aplastic anemia (160). In patients with severe neutropenia (ANC <500/uL), it is important to promptly evaluate and treat possible infection with empiric broad spectrum antibiotics. Patients who have received substantial amount of red blood cell transfusion develop organ dysfunction secondary to transfusion-associated iron overload. Effective iron chelation regimens are available both parenterally (deferoxamine) and orally (deferasirox) (161-163). Expert consensus has proposed to initiate chelation therapy when the hepatic iron concentration reaches 6–7 mg/g, dry weight, or when the patient has received approximately 100-300 mL/kg of transfusions (21, 27). As a surrogate marker, a serum ferritin level of 1000–1500 ug/L is used as the cutoff to start iron chelation, although serum ferritin levels may unreliably reflect total iron burden (164). Other parameters of iron stores, such as MRI imaging T2 and T2*, may also be applied clinically (164-166).

## CONCLUSION

Bone marrow failure syndromes comprise a minority of hypoproliferative anemia in clinical practice, not only for general internists but also for the practicing hematologist. Nevertheless, it is important that clinicians recognize bone marrow failure syndromes and refer patients to a specialist, because timely and optimal treatment may affect the prognosis. As briefly introduced in this review, recent advances in the understanding of pathophysiology have provided us insights into immune-mediated disease mechanisms, hematopoiesis maintenance, and cancer predisposition. Further laboratory and clinical research should improve clinical practice and provide opportunities to address fundamental scientific questions.

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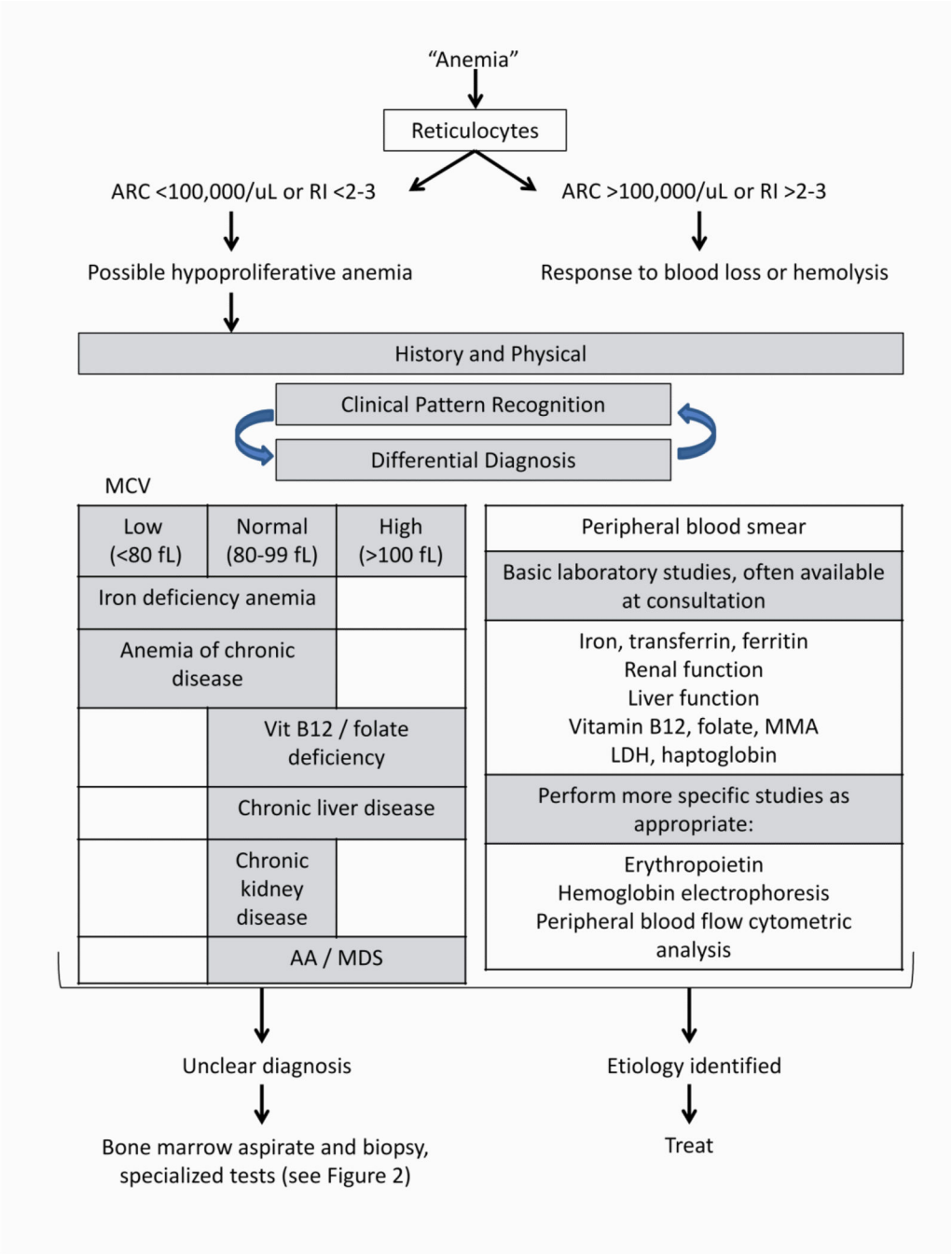


**Figure 1.**

Diagnostic approach to hypoproliferative anemia. ARC: absolute reticulocyte count. RI: reticulocyte index. AA: aplastic anemia. CKD: chronic renal disease. MDS: myelodysplastic syndrome. MMA: methylmalonic acid. LDH: lactate dehydrogenase

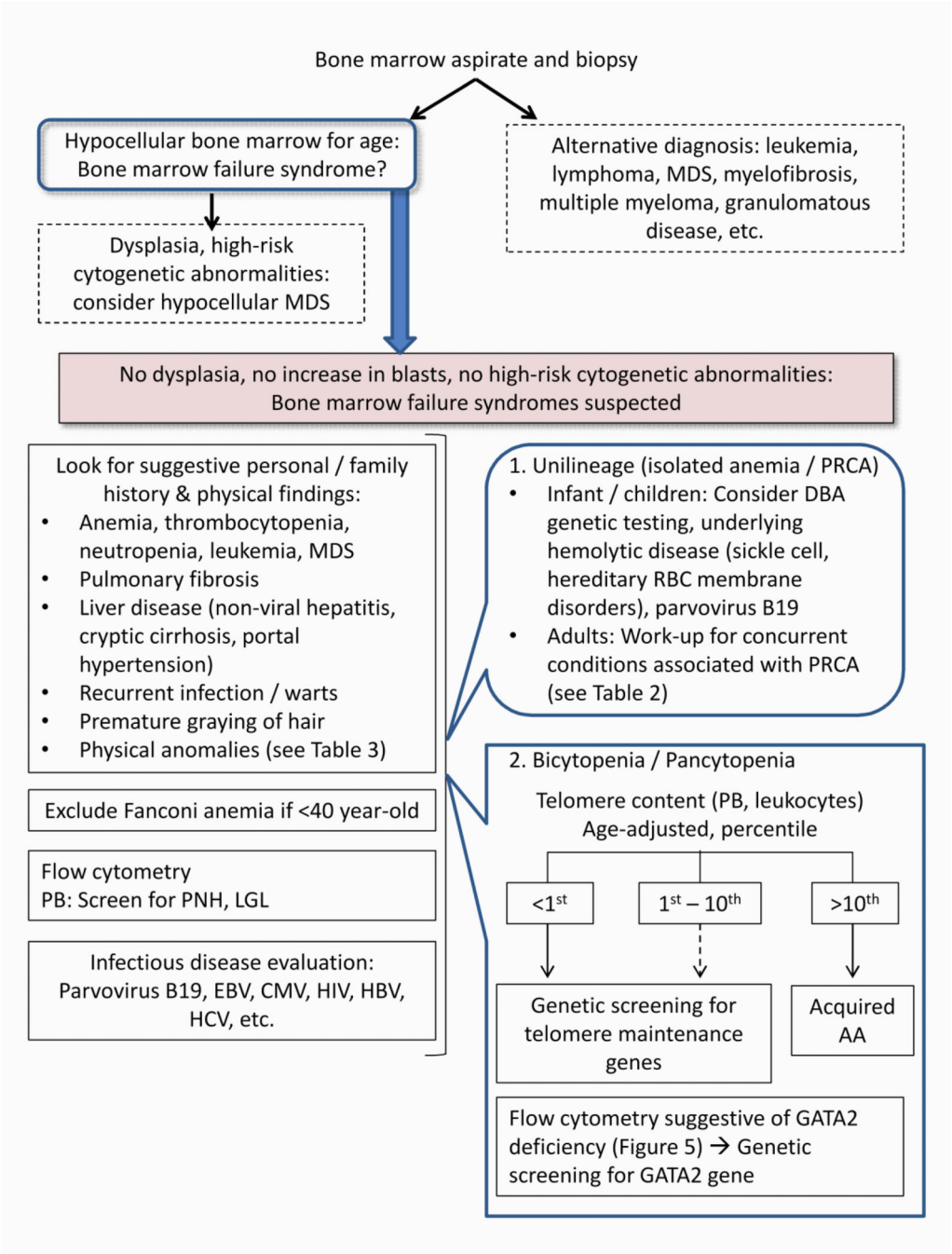


**Figure 2.**

Diagnostic approach to bone marrow failure syndromes. MDS: myelodysplastic syndrome. PNH: paroxysmal nocturnal hemoglobinuria. LGL: large granular lymphocytes. PRCA: pure red cell aplasia. RBC: red blood cell. PB: peripheral blood. AA: aplastic anemia

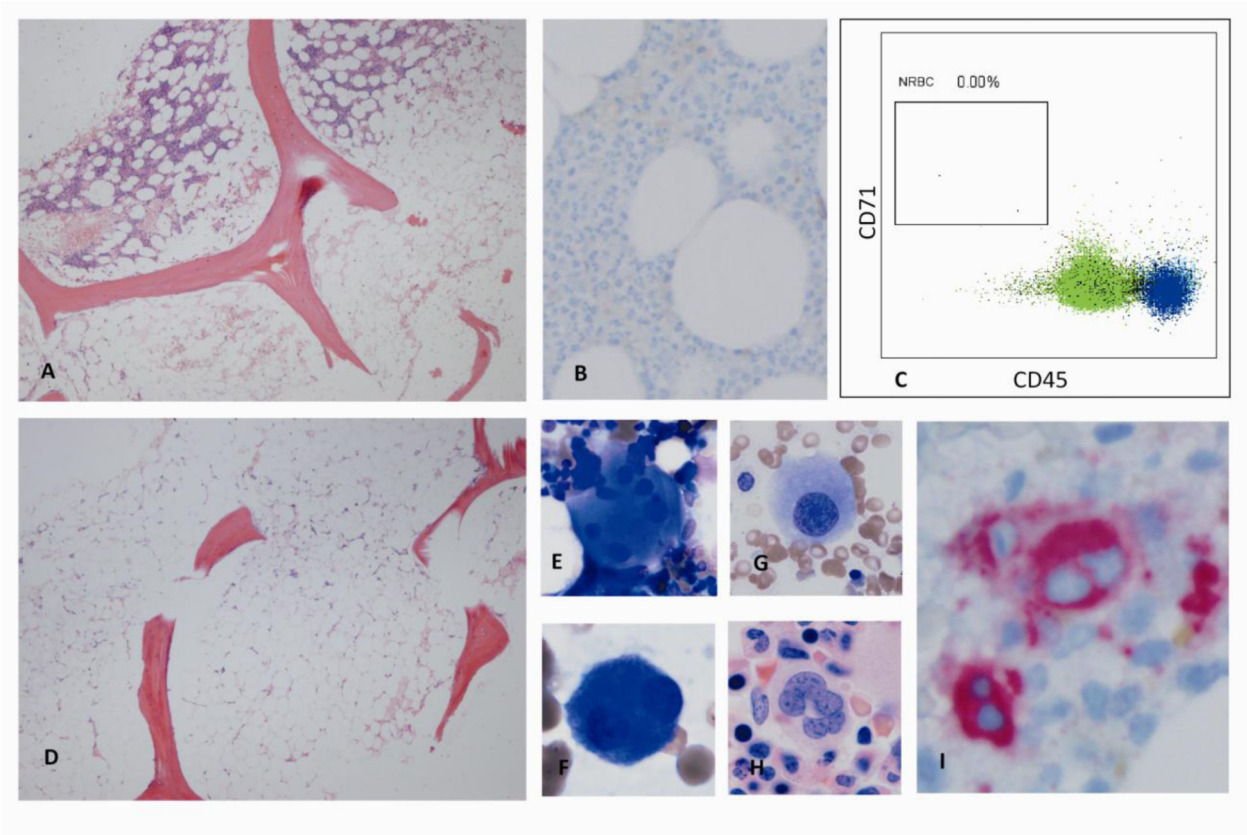


**Figure 3.**

A. Variably hypocellular marrow in aplastic anemia (cellularity <5% to 40%). B. Bone marrow biopsy of a patient with pure red cell aplasia (CD71 stain), revealing lack of erythroid precursors. C. Flow cytomery of bone marrow cells from a patient with pure red cell aplasia. Absence of erythroid lineage is confirmed and quantified. D. Typical bone marrow biopsy in severe aplastic anemia: hypocellular bone marrow replaced with fat. E-G. Examples of atypical megakaryocytes (E: Widely separated lobes without strand. F: small bilobated megakaryoctes. G: small monolobated megakaryocyte). H. Normal megakaryocte for comparison. I. Bone marrow biopsy of a patient with hypocellular MDS. Immunohistochemical staining for CD61 highlights atypical bilobated megakaryoctes. (Figure 3B-C: Courtesy of Dr. Raul Braylan)

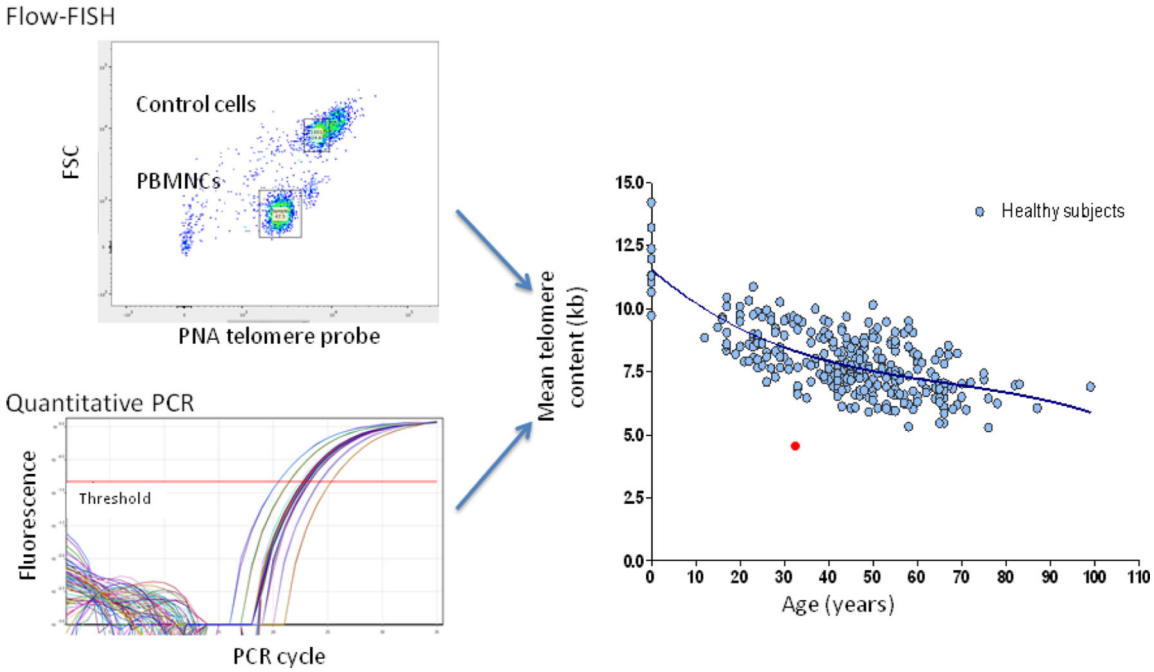


**Figure 4.**

Telomere content of leukocytes measured by standardized flow FISH or quantitative PCR. A patient's result is compared to age-adjusted normalized values. Calculated telomere length of total mononuclear cells below the first percentile for age strongly suggests a diagnosis of telomere disease. (Courtesy of Dr. Bogdan Dumitriu)

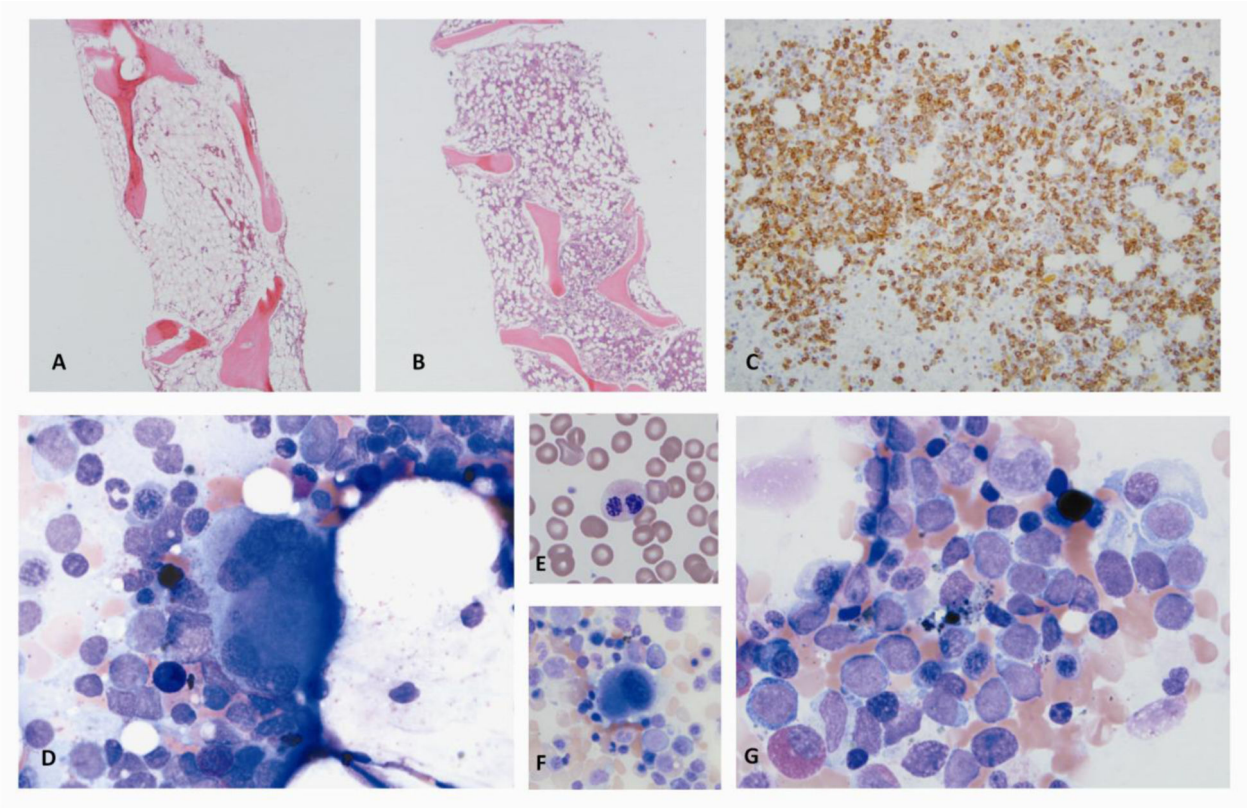


**Figure 5.**

GATA2 deficiency in the clinic: The patient presented as 18 year-old male with pancytopenia and marrow of AA, and within two years, he developed AML with myelodysplastic morphology.

A. Initial bone marrow biopsy at presentation: hypocellular marrow with trilineage hypoplasia compatible with AA. B-E. Bone marrow biopsy two years later: B. AML with myelodysplastic morphology; 30-40% cellularity. C. CD34 immunohistochemistry of biopsy, highlighting increased blasts. D. Dysplastic large osteoclast-like megakaryocyte with separated nuclear lobes, on aspirate smear. E. Pelgeroid PMN, peripheral smear. F. Small mononuclear megakaryocyte, on aspirate smear. G. Increased blasts, 35% on 500 cell differential of aspirate smear. (Courtesy of Dr. Danielle Townsley and Dr. Katherine Calvo).

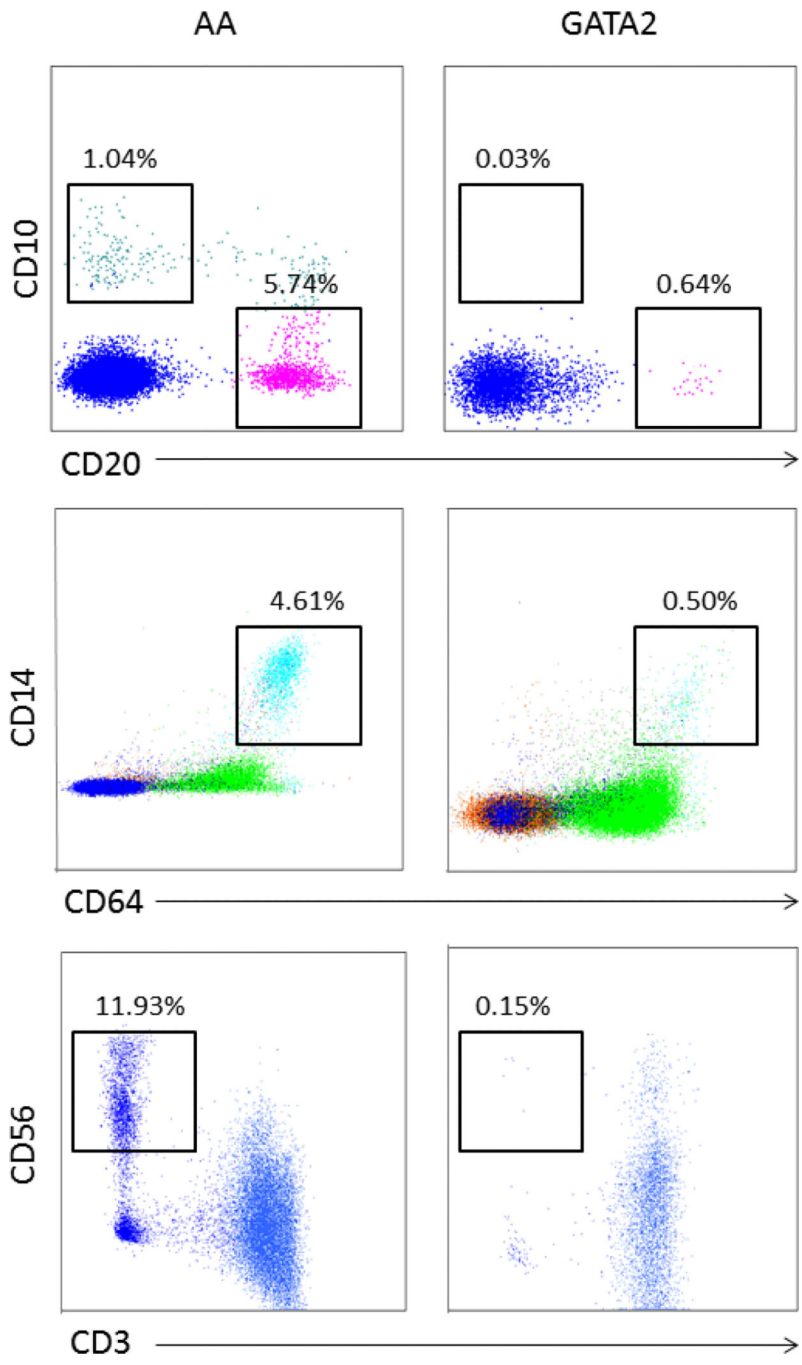


**Figure 6.**

Bone marrow flow cytometry of lymphoid subsets and monocytes in GATA2 patients. Compared to AA patients, GATA2 patients have disproportionately reduced bone marrow mature B cells (CD10−, CD20+), hematogones (CD10+, CD20−), monocytes (CD14+, CD64+), and NK cells (CD3−, CD56+) (Courtesy of Dr. Katherine Calvo).

**Table 1**

Classification of hypoproliferative anemia

| |
|---|
| Nutritional deficiency |
| • Iron |
| • Vitamin B12 |
| • Folate |
| Anemia of chronic disease and inflammation |
| Anemia of renal disease |
| Exposure to toxins or drugs |
| • Alcohol |
| • Drugs |
| Endocrine abnormality |
| • Hypothyroidism |
| • Hyperthyroidism |
| • Panhypopituitalism |
| • Primary or secondary hyperparathyroidism |
| • Androgen deficiency (male) |
| Hemophagocytic lymphohistiocytosis |
| Secondary to hematologic malignancies |
| • Leukemia |
| • Lymphoma |
| • Multiple myeloma |
| • Myelodysplastic syndrome (MDS) |
| • Myeloproliferative neoplasms |
| Bone marrow failure syndromes (see Table 2) |
| Myelophthisic anemia |
| • Metastatic cancers |
| • Hematologic malignancies |
| • Infections |
| • Autoimmune diseases |
| • Granulomatous diseases |
| • Renal osteodystrophy |
| • Hypo- and hyperparathyroidism |

**Table 2**

Classification of bone marrow failure syndromes

| Acquired | Constitutional |
|---|---|
| **Isolated anemia: PRCA** | |
| Transient | Diamond-Blackfan anemia |
| • Transient aplastic crisis, parvovirus B19 | Inherited sideroblastic anemia |
| • Transient erythroblastopenia of childhood | Congenital dyserythropoietic anemia |
| Infection | |
| • Persistent parvovirus B19 infection (immunocompromised patients) | |
| • EBV, CMV, HIV, hepatitis viruses | |
| • Other infections | |
| Malignancies | |
| • Thymoma | |
| • LGL leukemia | |
| • CLL | |
| • Other hematologic and solid malignancies | |
| Drugs and chemicals | |
| Pregnancy | |
| Complication of major ABO-mismatch HSCT | |
| Erythropoietin antibody due to usage of recombinant human erythropoietin | |
| Idiopathic | |
| **Bicytopenia / Pancytopenia** | |
| Radiation | Fanconi anemia |
| Drugs and chemicals | Telomere diseases |
| • Cytotoxic drugs (chemotherapy) | • Dyskeratosis congenita |
| • Benzene | • Telomeropathies |
| Infections | GATA2 deficiency |
| • EBV | Schwachman-Diamond syndrome |
| • Hepatitis viruses | |
| • HIV | |
| • Parvovirus B19 | |
| Immune disease / Malignancies | |
| • LGL leukemia | |
| • Thymoma | |
| • PNH | |
| • Collagen vascular diseases | |
| Pregnancy | |
| Idiopathic | |

## Table 3

Characteristics of constitutional bone marrow failure syndromes that accompany hypoproliferative anemia

| Disease | Genetics | Median age at diagnosis (range)* | Associated physical anomalies | Diagnostic tests (other than gene sequencing) |
|---|---|---|---|---|
| DBA | Ribosomal protein genes | 3 months (0-64) | Abnormal thumbs, short stature | Elevated erythrocyte adenosine deaminase, elevated Hgb F expression |
| Fanconi anemia | Fanconi anemia genes (DNA repair and genomic stability) | 6.5 (0-49) | Skin pigment changes or café-au-lait spots, short statrure, upper limb anomalites, microcephaly, renal malformations, hypogonadism, ear anomalities and deafness | Increased chromosomal breakage of peripheral blood cells after exposure to the DNA crosslinking agent (DEB, MMC) |
| Telomeropathy | Telomere repair complex or telomere protection complex (TERT, TERC, and others) | 14 (0-75) | Oral leukoplakia, dystrophic nails, skin hypo/hyperpigmentation (lacey reticular rash), pulmonary fibrosis, cryptic cirrhosis, portal hypertension, premature graying of hair | Short telomere contents of peripheral blood leukocytes (flow-FISH, quantitative PCR) |
| GATA2 deficiency | Hematopoietic transcription factor | Insufficient data | Recurrent warts, disseminated non-tuberculous mycobacteria infection, lymphedema, panniculitis, pulmonary diffusion and ventilatory defects, pulmonary alveolar proteinosis | Flow cytometry: peripheral blood - disproportionately reduced monocytes, B cells and NK cells, bone marrow - reduced monocytes, B cells, NK cells, and absent hematogones compared to AA |

*Reference 8